A Modified Method for Determining Available Lysine in Protein Recovered from Heat Treated Potato Juice

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Received July 16, 1975; accepted February 13, 1976

A dialysis pretreatment to remove low molecular weight interfering compounds from potato protein water prior to measuring its lysine availability is described. After dialysis the ε-trinitrophenyl-L-lysine showed excellent correlation with the magnitude of heat treatment the protein water received. Conversely, analysis of the same sample without pretreatment yielded erratic results.

In studies of some of the waste disposal problems facing the potato-starch manufacturer, efforts were directed toward recovery of heat coagulable protein or concentration of the entire effluent by thermal evaporation followed by spray drying (1,2). In either effort determination of the nutritional adequacy of the recovered protein was considered essential. Carpenter (3) observed a good correlation between the biological value of foodstuffs and the available lysine content of the protein contained therein. His chemical method for measuring the physiologically available lysine utilized 1-fluoro-2,4-dinitrobenzene. Kakade and Liener (4) subsequently reported on a simpler procedure for determining lysine availability in protein which involved a reaction with 2,4,6-trinitrobenzene sulfonic acid (TNBS).

Several workers (5–9), however, have reported that both methods are subject to interferences by carbohydrates resulting in low or variable recovery of the derivatized lysine, especially in vegetable protein. Similar variability was observed when the TNBS method was applied to potato protein water. Interfering substances reportedly have been removed by ion-exchange column chromatography (5,6) or canceled by applying a series of correction factors (9). Because of the complex and variable composition of potato protein water, we did not consider it practical to formulate correction factors. Instead, we removed the potential interferences such as sugars and hexosamines by dialysis prior to formation of the TNBS derivative.

In this study, data are presented which indicate the utility of the dialysis modification. Although small molecular weight peptides and free amino acids have been removed, the available lysine content of the protein remaining in the nondialyzable fraction is a reliable indicator of the total effect of heat processing of potato protein water.

MATERIALS

A Hitachi Perking-Elmer[1] Model 139, uv-visible spectrophotometer was used to measure absorbance.

ε-Trinitrophenyl (TNP)-L-lysine hydrochloride was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Picrylsulfonic acid, sodium salt dihydrate (TNBS) was purchased from Aldrich Chemical Company, Inc., Milwaukee, Wis.

Sodium bicarbonate, anhydrous ether, and hydrochloric acid were all Baker Analyzed Reagent Grade. Dialysis tubing, ¼ in. diameter inflated (catalog No. 3787-012), purchased from Arthur H. Thomas Company, Philadelphia, Pa., was used throughout this study. The pore size of this dialysis tubing has a molecular weight cutoff of 12,000.

METHODS

Potato protein water was prepared as described by Strolle *et al.* (1). Concentrates were prepared by evaporation in a forced circulation evaporator.

Potato protein powder was obtained by drying concentrates in a pilot plant-scale spray dryer.

Total protein nitrogen in the potato protein water was determined by the standard AOAC semimicro Kjeldahl method (10). The protein was isolated from the undialyzed sample by precipitating with 15% trichloroacetic acid plus a few drops of 0.1% sodium tungstate, or as the nondialyzable fraction after 24 hr dialysis. This total protein nitrogen value is the basis on which the micromoles of total and available lysine are expressed.

To establish the maximum level of lysine in the potato protein water, total lysine was determined by the Moore and Stein procedure (11) using an automatic amino acid analyzer. The potato protein water samples, before and after dialysis, were acid hydrolyzed by the method of Gordon and Basch (12).

ANALYTICAL PROCEDURE

The samples of potato protein water (3–4% total solids) were prepared for available lysine analysis by transferring approximately 2.5 g to a dialysis tube with a small quantity of deionized water and dialyzing for 24 hr. Usually three dialysis tubes were prepared, one to be used for lysine determination in triplicate and the other two for the determination of total nondialyzable nitrogen. After dialysis the contents of one tube were transferred quantitatively to a 50-ml volumetric flask with 4% sodium bi-

[1] Use of a company or product name by the Department does not imply approval or recommendation to the exclusion of others which may also be suitable.

carbonate solution, pH 8.5, made to mark with bicarbonate and allowed to extract overnight. After filtration through Whatman No. 50 filter paper, three 1-ml aliquots were analyzed as described by Kakade and Liener (4), with the following modifications: The concentration of TNBS was increased to 0.2% and the TNP–N–terminal amino acids or peptides were removed by extracting five times with 5 ml ethyl ether.

The contents of the other two dialysis tubes were transferred quantitatively to semimicro Kjeldahl flasks and analyzed for total nitrogen by the standard AOAC method (10).

The available lysine in potato protein water was determined before and after dialysis of the sample. The results were calculated as micromoles/100 g of original sample. Having determined the total protein nitrogen on the same basis, the respective lysine concentrations were recalculated as micromoles/100 mg total protein nitrogen. Reliability of this procedure was tested by triplicate analyses of a single sample on separate days. The mean value for available lysine was 287 micromoles/100 mg total protein nitrogen with a standard deviation of 6.84.

Potato protein water concentrate (30–45% total solids) was reconstituted to about 3% solids prior to sampling for dialysis and analysis.

Spray-dried potato protein powder was made into a slurry, transferred to dialysis tubes, and analyzed as above after overnight extraction with sodium bicarbonate solution.

RESULTS AND DISCUSSION

A typical potato protein water sample, exposed to temperatures of 60 and 71°C for 0, 1, and 60 min was analyzed for total and available lysine

TABLE 1

LYSINE CONTENT OF POTATO PROTEIN WATER BEFORE AND AFTER DIALYSIS

		Undialyzed			Dialyzed		
Temperature (°C)	Time (min)	Total protein nitrogen (mg %)	Total	Available	Total protein nitrogen (mg %)	Total	Available
			Micromoles lysine/100 mg nitrogen			Micromoles lysine/100 mg nitrogen	
60	0	36[a]	1034[b]	680[b]	40[c]	325[d]	254[d]
	1	32	1090	475	37	324	227
	60	31	1198	570	40	322	92
71	0	30	1119	678	40	354	271
	1	21	1095	609	30	340	122
	60	40	984	367	45	367	47

[a] Trichloroacetic acid precipitated protein.
[b] Based on total nitrogen in TCA precipitated protein.
[c] Total nitrogen after dialysis.
[d] Based on total nitrogen after dialysis.

TABLE 2

EFFECT OF PROCESS TEMPERATURE ON AVAILABLE LYSINE CONTENT OF POTATO PROTEIN WATER BEFORE AND AFTER DIALYSIS

Concentrate number	Total solids (%)	Micromoles/100 mg total protein nitrogen			
		60°C		71°C	
		Undialyzed	Dialyzed	Undialyzed	Dialyzed
1[a]	4	190[b]	258[c]	662[b]	277[c]
2	10	517	164	253	61
3	20	518	127	411	69
4	25	462	133	250	59
5	32	541	107	395	26
6	40	480	116	424	59

[a] Original, unheated solution.
[b] Based on total nitrogen in TCA precipitated protein.
[c] Based on total nitrogen after dialysis.

as previously described. Results (Table 1) indicate that the undialyzed samples were erratic, showing little or no correlation with the temperature and time of heat exposure. For example, 1-min heating at 60°C had less available lysine than the same sample heated at 71°C for 1 min. After 60 min at 60°C the concentration of this amino acid increased while an identical heating period at 71°C resulted in apparent loss of only half the original available lysine. These data are inconsistent with the known susceptability of lysine to heat damage, especially in the presence of carbohydrate.

By contrast, utilization of the dialysis modification to remove small molecular weight interfering compounds gave results (Table 1) which were

TABLE 3

TIME–TEMPERATURE EFFECT ON LYSINE AVAILABILITY

Time (min)	Micromoles available lysine/100 mg nitrogen[a]	
	60°C	71°C
0[b]	307	327
0	282	138
2	202	74
3	228	61
5	193	55
10	179	61
15	166	69
30	124	29
60	136	40

[a] Protein nitrogen (dialyzed) averaged 0.74% (w/w); SD = .0026.
[b] Does not include warm-up time nor cool-down time.

more consistent with expected lysine levels after the respective heat treatments. Although almost 70% of the total lysine was removed by dialysis, analysis of the remaining protein, i.e., the nondialyzable fraction, gives a fairly accurate indication of the effect of heat on lysine availability within the protein molecule. Initially nearly 80% of the total lysine was present as available lysine. After 1 min heating at 71°C about 65% of the total lysine was unavailable. One hour heating at 60°C was required to effect the same reduction in availability, while heating at 71°C for this period of time reduced the available lysine almost 90%. It is presumed that the epsilon amino group of the small peptides and free amino acids removed by dialysis also would have exhibited the same reactivity with the carbohydrate components of the sample.

The dialysis modification was further tested by analyzing a series of potato protein water concentrates prepared at 54.5, 60.0, 65.5, and 71°C. Samples were withdrawn from the evaporator at intervals, total solids were determined, then each sample was diluted to approximately 4–5% solids prior to analysis for available lysing "as is" and after dialysis. Although similar results were obtained with all four heating experiments, only the data for 60 and 71°C are shown in Table 2. The lack of any consistency in the data obtained from the undialyzed samples is obvious (Table 2), thus indicating that the method "as is" is inadequate for measuring the effect of heat exposure on lysine availability within the potato protein. Conversely, the data obtained after dialysis of the same samples show a consistent relationship between evaporation temperature and the measured available lysine content of the nondialyzable protein.

To determine more precisely the effect of time and temperature on the formation of a complex at the epsilon amino group of lysine, a carefully controlled study was conducted. A potato protein water (4% solids) sample was heated at both 60 and 71°C for 0 to 60 min. Results of this study are shown in Table 3. At 60°C the decrease in lysine availability was found to be gradual over the entire heating period. At 71°C, however, an appreciable quantity of the original available lysine was found to be chemically inactive almost immediately. In both studies the total nitrogen content of the nondialyzable fraction showed little variation (mean = 0.074% (w/w); SD = 0.0026).

SUMMARY

Low molecular weight compounds have been shown to interfere with the Kakade and Liener method for determining available lysine in potato protein recovered from starch manufacturing plants. A dialysis pretreatment was found suitable for removing these compounds prior to analysis. By utilizing this modification the value for available lysine content of the recovered protein was consistent with the magnitude of heat treatment

during processing of the potato protein water. Thus, process optimization to ensure recovery of nutritionally adequate (high available lysine) protein is possible.

ACKNOWLEDGMENTS

The authors thank Dr. William G. Gordon for determining total lysine content of the potato protein and Mr. Nicholas C. Aceto for his encouragement during this research study.

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